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Study the Harmful Effects of Indomethacin in Liver of Male Albino Rats and Role of the Nano-Extract of *Cardia Myxa* Fruits in Preventing

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Abstract

The current study was conducted in the animal house-College of Science / University of Kufa. The study aimed to reveal the protective role of nano-extract of the fruits of the *cardia myxa* plant on the histological composition of the kidneys of male white rats treated with indomethacin, their ages ranged between (8-10) weeks and their weights ranged between (200-250) gm, then the animals were divided into (8) groups, each group contained (5) male rats and were as follows: the first group represented the control group which was dosed with physiological saline only, while the the second group is the group treated with indomethacin at a concentration of 0.143 mg/kg, while the third and fourth groups were the group treated with nanoextract at two concentrations (20 and 30 mg/kg, respectively, While the fifth group was the group that dosed zinc oxide, Zno, at a concentration of (1 mg), and the sixth and seventh group represented the group treated with the nanoextract at two concentrations (20 and 30 mg/kg) + the drug Indomethacin at a concentration of (0.143) mg/kg respectively, while the eighth group was treated with the plant extract of the fruits of the Panemer plant, 500 mg/kg + indomethacin at a concentration of (0.143) mg/kg respectively. Male rats were dosed in all groups for a period of (40 days) at the rate of one dose per day through a gastric dosing tube, after which the animals were sacrificed and microscopic sections of the liver were made for histological study. The results showed in the control group the normal tissue of the liver, as it was the normal histological structure of the liver, while the results for the drug group showed the presence of pathological changes in the liver tissue represented by the presence of necrosis and severe hemorrhage, while the histological results of the two nanoextract groups with two concentrations (20 and 30 mg / kg), respectively It showed the normal shape of the hepatocytes and the regularity of the hepatic cords. As for the results of the group treated with zinc oxide (Zno), it revealed harmful effects to the liver tissue, such as severe necrosis of the cells and the expansion of sinusoids, while the results of the sixth and seventh groups, treated with two concentrations of nano-extract + indomethacin drug, showed that the structure of the liver was close to normal tissue with little change in hepatocytes and regular hepatic cords, as for the results of the eighth group, the plant extract group of the fruits of the panamer plant of C.myxa at a concentration of 500 mg / kg + the drug at a concentration of (0.143) mg / kg, it was observed that slight changes occurred in the liver tissue, such as necrosis in some liver cells and degeneration of some of them.

Key words: indomethacin, liver , albino rats nano-extract, *Cardia myxa*, fruits .

Introduction

The *Cardi myxa* plant is locally called (Bumber) and it is one of the largest species belonging to the borage family Borginaceaegd. It is a flowering plant with a length of 12 m,its fruits are spherical or oval, the plant contains chemical compounds in its fruits, seeds and all parts of the plant (Abdel – El Ameen *et al*., 2017). This plant has been known since ancient times for its high medical benefits and its use in medicine in all its parts, bark, leaves, fruits, seeds and it contains inhibitory compounds and secondary receptors such as alkaloids, phenols, sterols, saponins, coumarins, phenolic acids, and others (Musa *et la*., 2020). Zinc oxide, ZnO, is an insoluble, insoluble, white powder with a high surface area, these properties make it an important agent for many applications and applications and ZnO is used in cosmetics, biosensing, electronics, and its high absorption of ultraviolet radiation. (Diez-pascual and Diez-vicenle, 2014). The indomethacin drug is one of the drugs that belong to the nonsteroidal drugs and is anti-inflammatory and one of the most used drugs in all parts of the world (Botlonni *et al*., 2009), indomethacin drug has been used since its discovery in 1963 to treat several diseases, including gout. , arthritis, myopathy, rheumatoid arthritis (Matsui *et al*., 2011), indomethacin drug is called by many commercial names such as Indocin, Indomin, Indocint, and Indocid (Botting, 2006) ,this drug is used as an analgesic for pain, antipyretic, arthritis, gout, osteoarthritis, and rheumatoid spondylitis (Matsul *et al*., 2011), the mechanism of action of this drug is similar to that of other nonsteroidal anti-inflammatory drugs that inhibit the biosynthesis of prostoglandins (PGS) by inhibiting the activity of enzymes known as cyclooxygenase (Cox) which exists mainly in two forms Clyco ocxygenase-1 (Cox1), and Clycooxygenase-2(Cox-2) (Smith *et la*., 2012), the use of this drug is accompanied by many side effects including the gastrointestinal effect which is considered one of the most harmful aspects and the gastro-intestinal tract is the main target of these drugs (NASIDs), especially indomethacin because it rapidly absorbs substances through the gastrointestinal tract when it is taken orally (Mayo *et al*., 2016). Nanotechnology is the manipulation of matter at molecular or atomic scales to build structures or systems. The term nanotechnology consists of two parts (nano) and technology. (Nano) is derived from the Greek scientist (nanos) means dwarf, either technology is a term that refers to the use or creation of modern technical means that have specific names such as billion (10-9) (Regers et al., 2014), that nanoscience is the science of the art and processing of matter on the nano level, the value of nano (Nano technology) deals with materials with very small nano sizes and will be called nano particles, One in the range (1-100) nanometers (Tjong and Chan,2004), the nanoparticles are manufactured in different ways and have professional characteristics. The nanoparticles are left in the way they are prepared, either a method from the smallest to the largest, and it is called the TDA Top-down approach. Top-down approach or bottom-up-approach (BuA) (Wang and Xia., 2004).

Materials And Methods of Work

Experimental animals:

In this experiment, (40) white rats of the *Rattus-rattus* species were used, their weights ranged between (200-250) g and their ages ranged between (8-9) weeks, The experiment was conducted from March 2022 until October 2022 in the animal house of the College of Science / Kufa University, the animals were placed in cages made of plastic covered with iron clamps to which water bottles were attached ,the cages were spread with wood shavings, and it was constantly changing. The animals were fed according to a natural diet suitable for them, with the preparation of laboratory conditions and lighting which consisted of 12 hours of darkness and 12 hours of light at a temperature of 25 C, then the animals were divided male rats, with (5) animals for each group, and the groups were as follows: The first group was the control group were given a 0.9% physiological solution, the second group was dosed with indomethacin at a concentration of (0.143) mg/kg, and the third group was ZnO group with a concentration of (1 mg / kg), and the fourth and fifth groups were dosed with the nano-extract at two concentrations (20 and 30) mg / kg respectively, and the sixth group was the group treated with the plant extract 500 mg / kg + the drug indomethacin, the seventh and eighth group was dosed with the nanoextract + drug group was where the concentration of the nanoextract was (20 and 30 mg/kg), respectively, and the drug was (0.143 mg/kg).

Preparing The Aqueous Extract Of *Cardia Myxa* Fruits:

The aqueous extract of the plant was prepared by adding (109) of the powder of the *Cardia myxa* plant fruits to (100ml) of distilled water in a glass flask and placed in a water bath at room temperature for two hours, the solution was left to cool and then filtered with paper, filtration and then centrifuge 4500 cycles / for 10 minutes, after which the extract is collected in a clean box and kept in the refrigerator until use (Inshad *et al*., 2020).

Preparation Of The Nano-Extract Of The *Cardia Myxa* Fruits:

The preparation of the nano-extract *of Cardia myxa fruits:* according to the method of (Inshed *et al*., 2020) with some modifications to it by adding proof of Zinc acetate, which has the chemical formula (Ch3Coo)2 Zn 2H2O at a concentration of 0.2l/ moL to 100Ml of plant extract at room temperature, naotl was added to neutralize the acidity function down to PH=7, then the mixture was placed on the hot plate for 3 hours at 60°C until the solution changed color, evidence of the formation of zinc oxide nanoparticles, after which it was filtered by a centrifuge, at 4600 cycles/min, the precipitate was taken, then washed (3) times, diluted in an electric oven, and tempered in an electric oven at a temperature of 40-45 m, then ground with an electric manual mill and kept in a clean box until use.

Preparation Of Indomethacin:

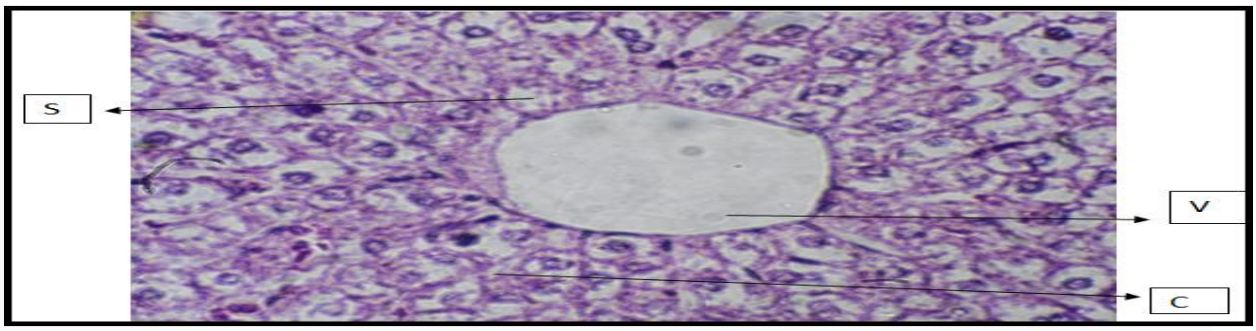
The drug was prepared from Al-Najaf pharmacies in the form of capsules, and the capsule contains 50 mg of the drug(Demasceno *et al*., 2008). The male rats were given a single dose for two days, according to body weight, for a period of (40 days), by means of an oral dosing tube and one dose per day, after the end of the experiment, the animals were sacrificed by anesthetizing them with (chloroform),then placing the animals on a dissecting spectrum and fixing them with pins to perform the dissection process and opening the abdominal cavity, after that the livers were extracted from all male rats and the fatty tissue and the connective tissues were removed and the livers were placed in a 10% formalin solution for the preparation purpose of histological study.

Results and Discussion

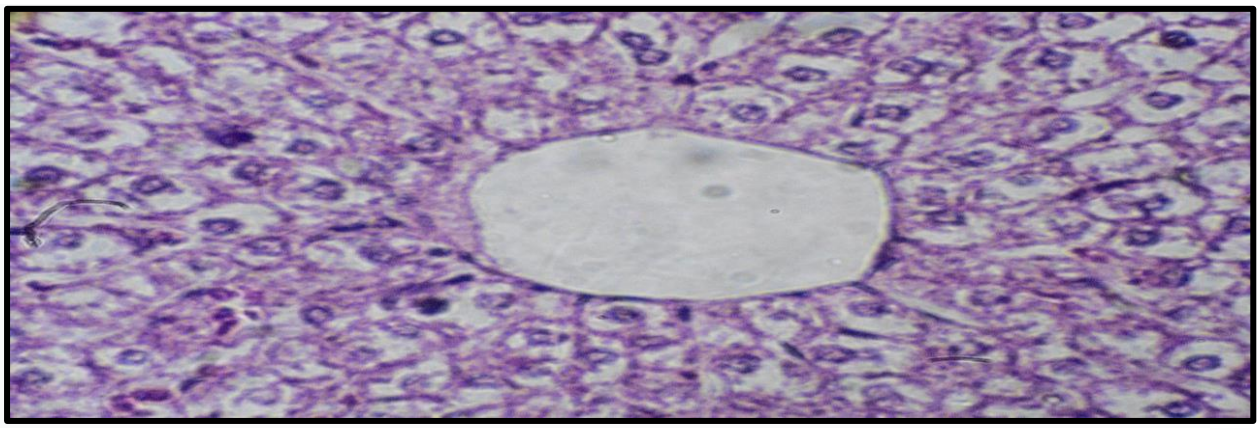
Microscopic examination of the liver tissue of male rats in the control group revealed the normal structure of the liver and the absence of any histological changes in it as in figure (1), while the results showed that the group of rats treated with indomethacin at a concentration of (0.143) mg / kg of body weight for a treatment period of (40)day .there are pathological changes in the liver tissue of rats such as widening of the central vein and its wall shattering, necrosis and degeneration of hepatocytes and expansion of sinusoids, destruction of liver tissue and hemorrhage in it, as in figure (2), and the reason for these results may be due to the fact that the drug indomethacin can have toxic effects on the histological structure to different organs including the liver by increasing the duration of the dose (Sebeeh and Al -Naimi *et la*., 2014), and some studies have shown that the indomethacin drug caused toxic effects on the male genital organs when they used the drug for a long period of time during dosing to animals, as the researcher noted (Han *et al*., 2016) when using the indomethacin drug caused atrophy and destruction of various tissues of the body and weight loss for animals during for a long time, this was shown by the researcher's study (Abdullah *et al (*2016) the effects on liver and kidney tissue when treated with indomethacin, some studies also revealed that when using indomethacin drug in toxic doses, it led to the occurrence of structural deformities before and after birth in newborns and this drug has a significant effect on the organs of rats when used for a long period of time (Al-Essawi, 2020), other studies reported that indomethacin caused abnormal effects on the concentrations of some biochemical and physiological blood parameters including albumin, erythropoietin, vitamin D3, calcium ions, malondialdehyde (MDA) ,RBCs, hemoglobin (Hb) concentration, and PCV , and the occurrence of histological changes in the liver tissue such as necrosis and degeneration of the hepatocytes, and the destruction of the central vein in pregnant female rats during different stages of pregnancy (Al-Essawi *et al*., 2020), as for the results of the third group, ZnO zinc

oxide with a concentration of (1 mg / kg), its results showed a strong effect on liver tissue with clear histological periods (Xia *et al*., 2017), as the liver is the main organ responsible for the metabolism of drugs and chemicals and this makes it a target and vulnerable to necrosis and inflammation of the liver as shown in figure (3), when it showed severe necrosis in the hepatocytes, bleeding and hemorrage in the hepatocytes, degeneration in the hepatocytes and this was shown by a study of (Alesawi,Z.F.H.; Alaridhi,J.A.M.(2022).) and a study of (Surekh *et la*., 2012).

The results of the microscopic examination of the two groups treated with the nano-extract at two concentrations (20 and 30) mg / kg of body weight respectively showed that the liver tissues were close to normal with the regularity of the hepatocytes as in the figures (3 and 4) respectively,and the reason for this result is due to the therapeutic properties of the drugs due to the presence of complex chemicals with a diverse composition in one or more parts of these plants (Alesawi,Z.F.H.; Alaridhi,J.A.M.(2022)), plant compounds are classified according to their composition in the form of alkaloids, helicosides, corticosteroids, essential oils, flavonoids (Farrell *et la*., 1975) and *Cardia myxa* fruits which was carried as a nanoextract is described as antitoxin, antimicrobial and antioxidant, this is due to the antioxidants contained in the plant as some studies revealed such as the study (Ranjbar *et la*., 2013) which showed the protective role of *Cardia myxa* plant extracts can be attributed to the antioxidant effect of the flavonoids contained in it, as for the results in the two groups treated with the nanoextract at two concentrations (20 and 30)mg / kg + indomethacin at a concentration of (0.143 mg / kg) of body weight for the period (40 days) showed a slight change in the hepatocytes and the regularity of the hepatic cords with a slight necrosis in the endothelium of the hepatocytes as in figures (7 and 8) respectively, and the reason for the current result may be due to the protective role of the nano-extract to the active compounds in the plant and phenolic compounds such as phenylbromatoids (Cupta *et la*., 2017) and his indicates that the plant generates free roots and is attributed to the defensive effects of the compounds present in the plant and its main active content (Albu *et al*.,1998), and this was shown by the study (Sheikndman and Karnkarn, 2014). Who mentioned in their study the role of some plant extracts in protecting against hepatic effects resulting from dosing animals with other drugs, the results of the histological microscopic examination of the structure of the liver in the group and the eighth group which were treated with aqueous plant extract of *Cardia myxa* fruits at a concentration of 500 mg/kg + indomethacin at a concentration of 0.143 mg/kg, showed minor damage represented by the occurrence of simple necrosis in the liver cells as in the figure (8) when was compared to the control group and the groups treated with the nanoextract is not associated with the drug that was given a protective role and this is what the researcher showed (Mojtab *et al*., 2019), where the extract of *Cardia myxa* fruits was used and at the same concentration (500 mg / kg), it showed slight effects and showed protective effects on the changes brought about by the biochemical factors of blood and liver tissues resulting from the harmful effect of Cdcl2 in male rats,a study (Pan *et al*., 2014) showed the protective effect of *Cardia myxa* extract of the protective effect of the plant and its components (Ceffic acid, Phytosterol, ceffic acid, rufin) on the liver tissue that it has antioxidant and anti-inflammatory effects that have a protective role and have a preservation role on liver tissues, and *Cardia myxa* plant extracts reduced bilirubin levels due to its protective effect on the liver and improving its functional capabilities (Jabbar & Noor 2018).



Figue (1) : Photomicrograph (Control negative)of a cross-section of the normal liver of a male rat from the negative control group showing: Normal hepatic architectures.(V) normal center vein (C) normal Hepatic cells , (S) normal Sinusoids.(Hematoxylin and Eosin.400x).



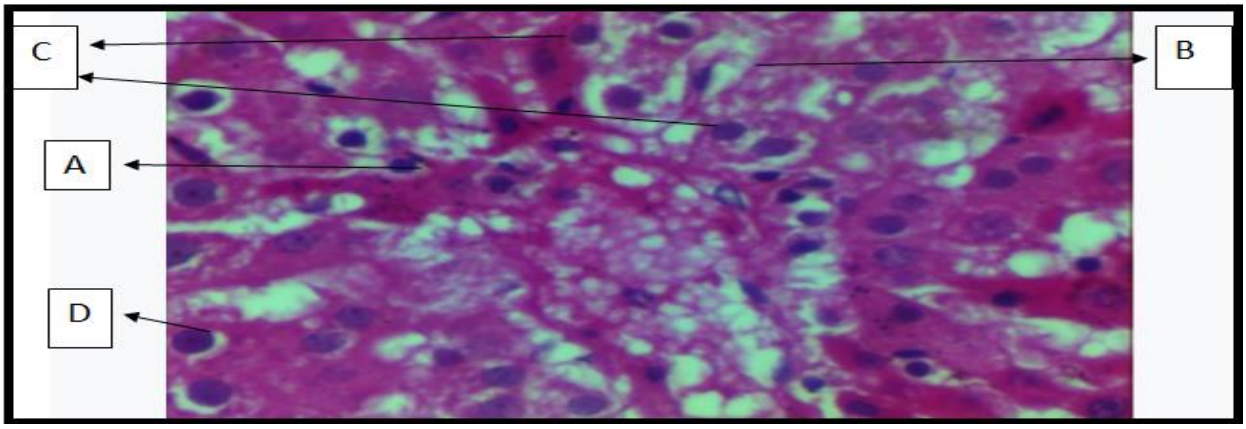
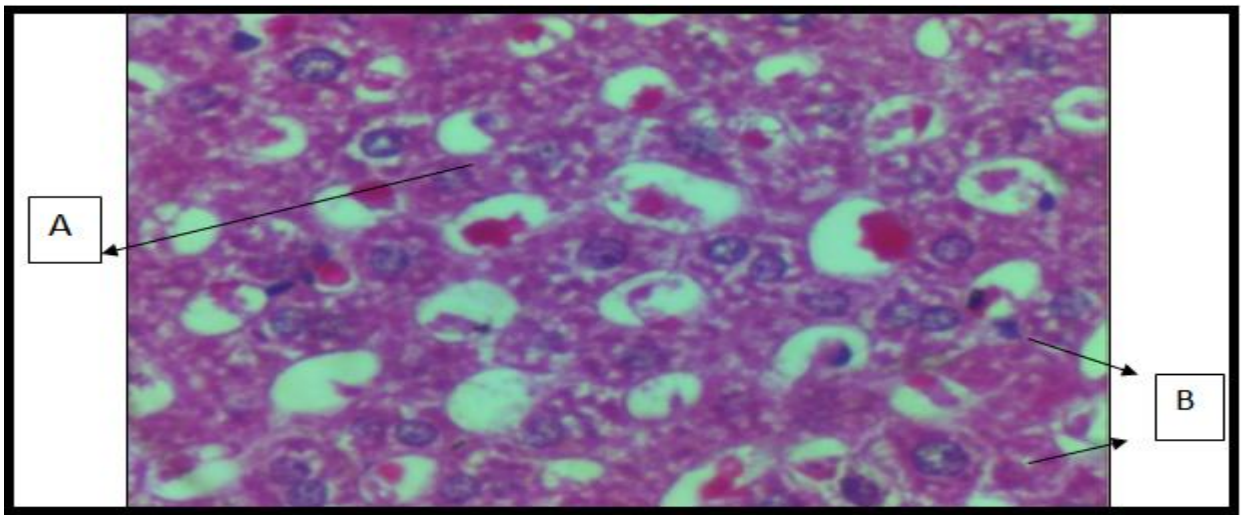


Figure (2) Photomicrograph of Control positive a cross-section of the liver of a male rat from the positive control group which treated with Indomethacin drug showing: (A) degeneration of hepatocytes (B)Necrosis of hepatocytes cells(c) inflammatory cells.(d) sinusoidal breadth .(Hematoxylin and Eosin.400x).



Figure(3) : Photomicrograph of a cross-section of the liver of a male rat from the positive control group which treated with oxide zinic showing: (A) degeneration of hepatocytes (B)necrosis of hepato cytes.(Hematoxylin and Eosin.400x).

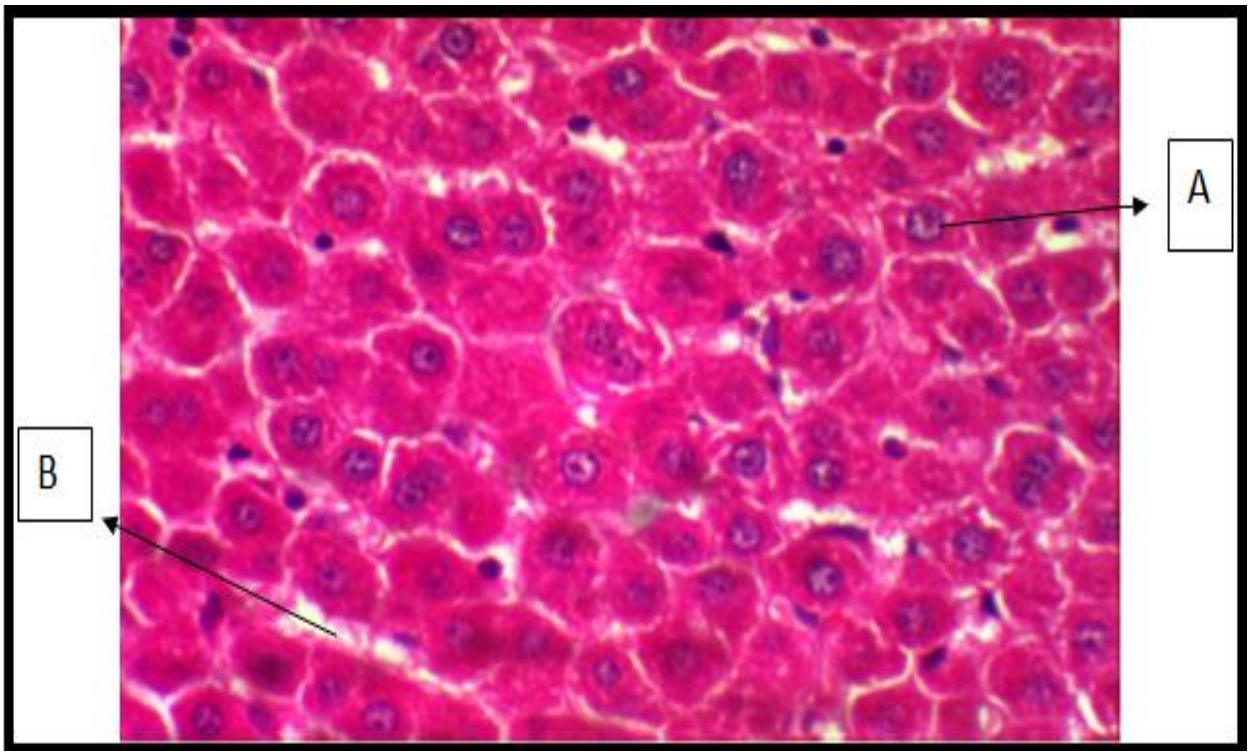


Figure (4): Photomicrograph of a cross-section of the liver of a male rat from the treated group with nano-extract at concentration of 20 mg / kg showing: (A)Normal hepatocytes(B) Mild breadth . (Hematoxylin and Eosin. 400X).

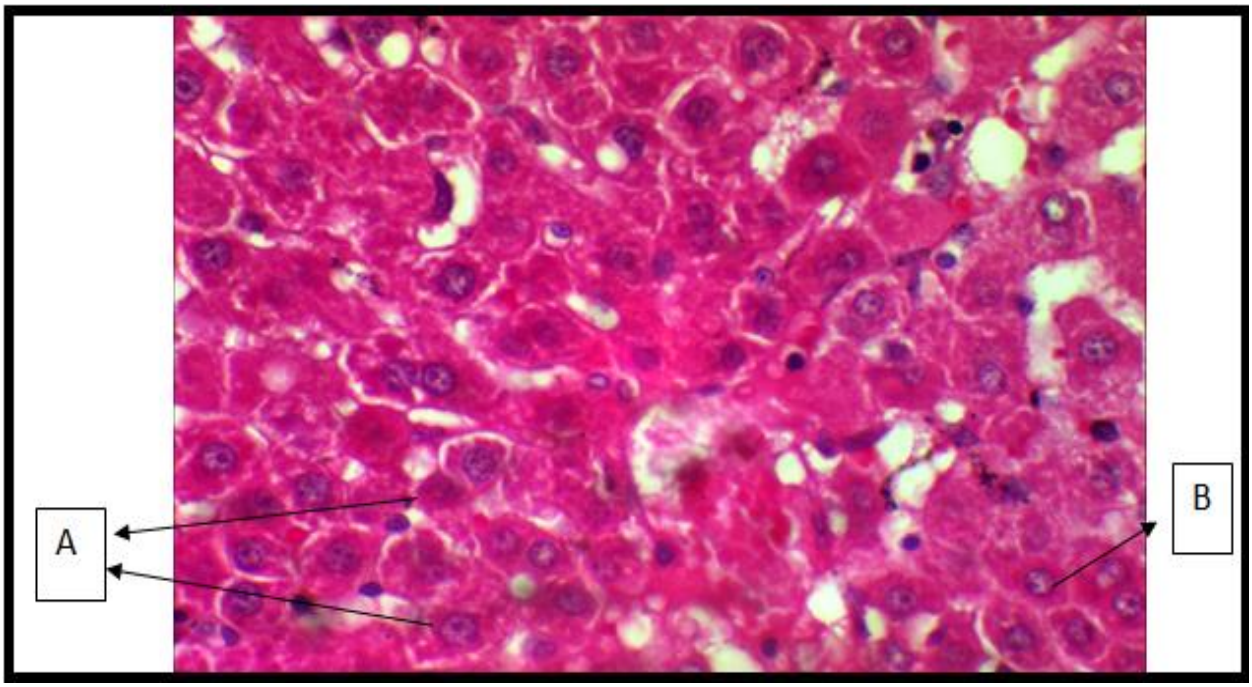
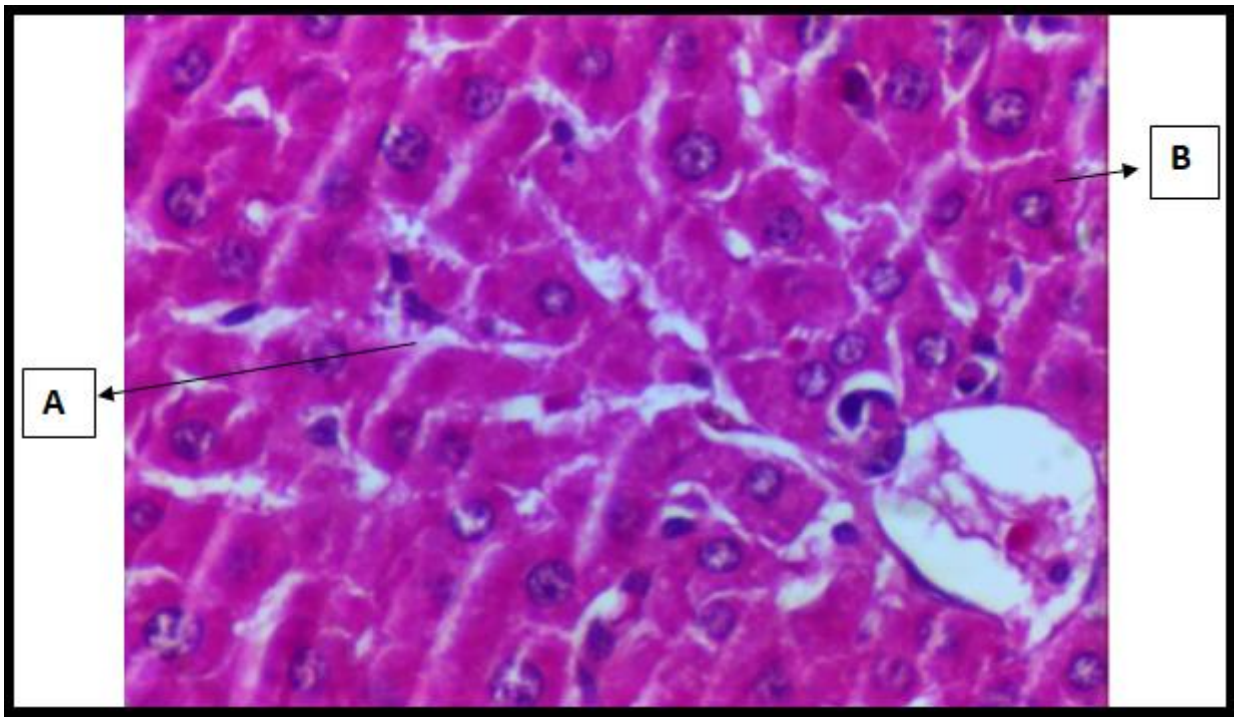
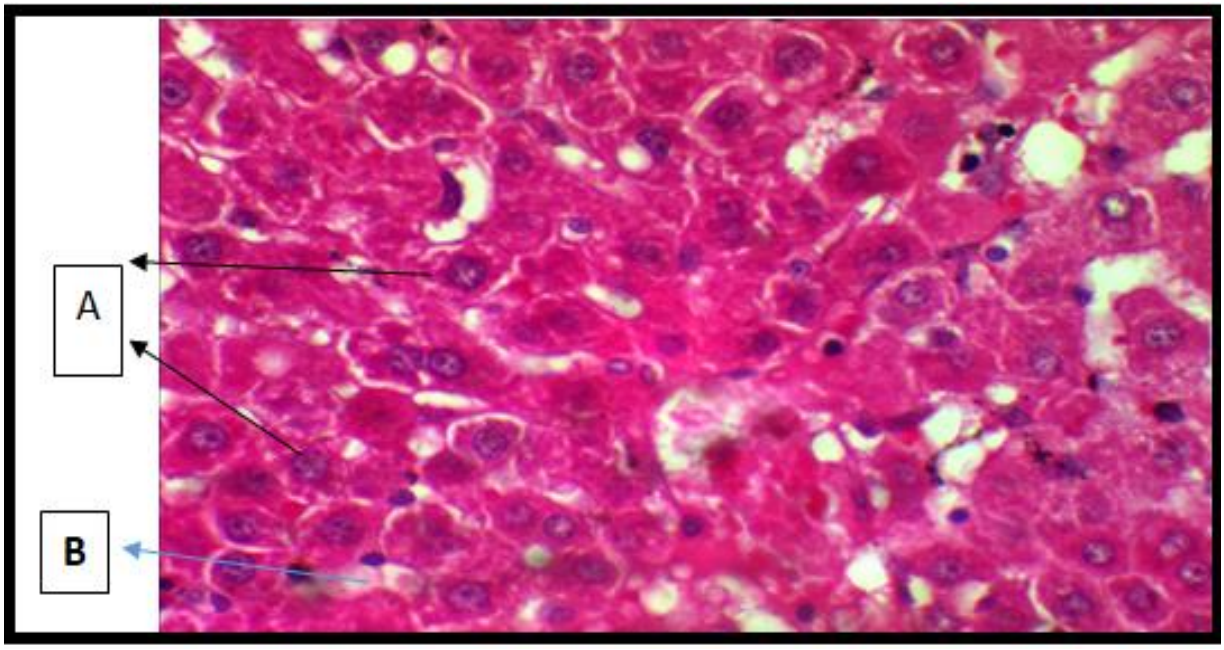


Figure (5): Photomicrograph of a cross-section of the liver of a male rat from the treated group with nano-extract at concentration of 30 mg / kg showing:(A) :mild (B) necrosis of individual hepatocytes observed in hepatic tissue.(Hematoxylin and Eosin. 400X).



Figure(6): Photomicrograph of a cross-section of the liver of a male rat from the treated group with nano-extract at concentration of 20 mg / kg+indomethacin drug at concentration of (0.143) mg / kg breadth.(Hematoxylin and Eosin. 400X) showing(A) Normal hepatocytes. (B)Mild



Figure(7): Photomicrograph of a cross-section of the liver of a male rat from the treated group with nano-extract at concentration of 30 mg/kg+indomethacin drug at concentration of (0.143) mg/kg showing: (A) Normal hepatic cells (B) mild Necrosis hepatic cells .(Hematoxylin and Eosin. 400X).

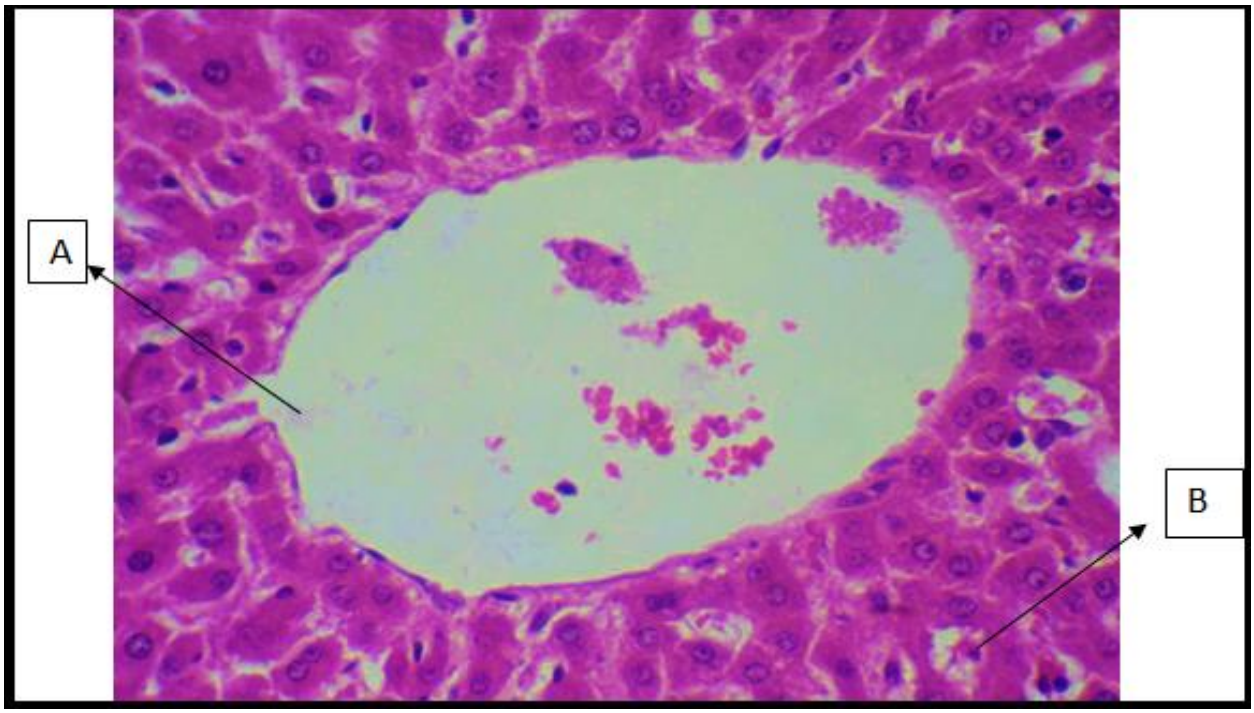


Figure (8) :Photomicrograph of a cross-section of the liver of a male rat from the treated group with plant extract of *Cardia myxa* fruits at concentration of 500 mg / kg+indomethacin drug at concentration of (0.143) mg / kg showing: (A) Normal vein central (B) mild Necrosis in hepatic cells little crash in his wall.(Hematoxylin and Eosin. 400X).

Conclusion : We conclude from the current study that the nano-water extract with different concentrations has a protective role in protecting the histological structure of the liver in male rats when treated with indomethacin.

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